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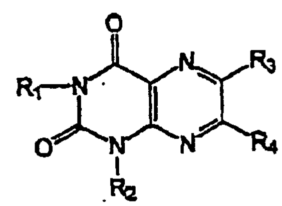

(I)

(57) Abstract

The invention relates to a pharmaceutical composition for the treatment of autoimmuno disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula (I) especially a lumazine optionally combined with a second active agent.

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IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES

The invention relates to a pharmaceutical composition for the treatment of autoimmuno disorders and/or the treatment or prevention of transplant-rejections comprising pteridine derivatives.

The invention further relates to combined pharmaceutical preparations comprising one or more pteridine derivates and one or more known immunosuppressant, and to a group of novel pteridine derivates as such.

Further the invention is also related to a method for the treatment of autoimmuno disorders and/or of transplant-rejections.

Several pteridine derivates are known in nature and used in the preparation of medicines, for example as described in EP-A-108 890. Other medical uses of derivatives of pteridine are described in WO 95-31987 as NO-synthase inhibitors, for example for the treatment of diseases caused by a high nitrogen monoxide level. Further, WO-95-32203 describes also the use of tetrahydropteridine derivatives as NO-synthase inhibitors.

Both above-mentioned WO publications disclose also the use of these specific pteridine derivatives in the treatment of pathologically low blood pressure, in particular septic shock and combined with cytokines in tumor therapy and in transplant-rejection diseases.

Although some of these pteridine derivatives are claimed as potentially active for the treatment of transplant-rejection diseases, direct evidence for their effectiveness is lacking. Thus there still is a need for specific and highly active immunosuppressive compounds, in particular immunosupressive compounds active in the cosignal pathway.

A first object of the invention is to provide a pharmaceutical composition having high immunosuppressive activity. Another object of the invention is to provide a

combined immunosuppressive preparation which causes a superadditive effect, comprising a pteridine derivative of the invention and other known immunosuppressants.

Another further object of the invention is to provide immunosuppressive compounds, which are active in a minor dose, in order to decrease the considerable treatment costs.

Known immunosuppressive compounds are for example cyclosporine A, subsituted xanthines, tacrolimus (FK 506), rapamycine (RPM), leflunomide, mofetil, adrenocortical steroids, cytotoxic drugs and antibody preparations.

The immunosuppressive effect of cyclosporine A (CyA) is already known since 1972. However, due to its nephrotoxicity and several other side effects CyA has not been able to establish itself as the optimal and final drug of choice.

Methylxanthines, for example pentoxifylline (PTX), are known having immunosuppressive effects in vitro.

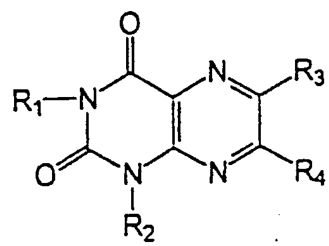
Recently (Lin Y. et al, Transplantation 63 (1997) it has been found that the co-medication of an immunosuppressive compound such as cyclosporine A (CyA) or FK506 or RPM (rapamycine) with a methylxanthine derivative, in particular A802715 (7-propyl-1(5-hydroxy-5-methylhexyl)-3-methylxanthine) leads to a superadditive increase in the immunosuppressive action.

Likewise, other substituted, in particular substituted 8-phenylxanthines have been found to possess immunosuppressive effects in vitro (application EP 98.201323.7).

The present invention relates in particular to the application of a group pteridine derivatives and their pharmaceutical salts, possessing unexpectedly desirable pharmaceutical properties, i.c. are highly active immunosuppressive agents.

The invention demonstrates the

immunosuppressive effects of pharmaceutical composition for the treatment of autoimmuno disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula:



wherein:

R_1 and R_2 are independently hydrogen; aliphatic saturated or unsaturated; straight or branched carbon chain with 1 to 7 carbon atoms; substituted or unsubstituted aryl or alkylaryl substituents, whereby the carbon atoms may be oxidized represented by alcohol or carbonyl function or carboxylic acids and their esters;

R_3 and R_4 are independently hydrogen, hydroxyl, halogen,alkyl, haloalkyl, alkoxy, wherein alkyl and the alkyl group may be branched or straight and contains one or four carbon atoms, formyl and derivatives such as hydroxylamino conjugates and acetals, cyano, carboxylic acids and carboxyl acid derivatives such as esters and amides, sulfhydryl, amino, alkylamino, cycloalkylamino, alkenylamino, alkynylamino, benzylamino, hydroxylalkylamino, morfolinoalkylamino, fenylhydrazino, morfoline, piperidine, mercaptobenzyl, mercaptoalkyl, cysteinyl ester, styryl, substituted or unsubstituted aromatic ring; aromatic or heterocyclic substituent substituted with an aliphatic spacer between the pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, whereby said spacer may contain an alcohol function, carbonyl function, halogen, ether, and may be saturated or unsaturated; branched or straight, saturated or unsaturated aliphatic chain of 1 to 7 carbon atoms

which may contain one or more functions chosen from the group comprising carbonyl, alcohol, ether, carboxyester, nitro, thioalkyl, halogen or a pharmaceutically acceptable salt thereof; and

X and Y are independently oxygen or sulfur or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier.

Preferred pteridine derivatives comprising compositions are given in claims 2-9. Particularly preferred are the compositions according to claim 10.

The invention further relates to a combined preparation having synergetic effects containing 1) cyclosporine A, substituted xanthines, tacrolimus (FK506), Rapamycin (RPM), Leflunomide, Mofetil, adrenocortical steriods, cytotoxic drugs and antibody compositions and 2) at least one pteridine derivative of formula (I) defined above, and optionally a pharmaceutical excipient, for simultaneous, separate or sequential use in (auto)immune disorders and/or in the treatment of transplant-rejections.

The invention further relates to a method for treating auto-immuno disorders or transplant-rejections in a subject by administering an effective amount of a pharmaceutical composition of claims 1-11, to the compounds as such as defined above, to the use of these compounds for the treatment of autoimmuno disorders and/or the treatment and/or prevention of transplant rejections, and to a method for selecting potent immunosuppressive agents based on the determination of the three parameters MLC, ACD_3 and ACD_{28}.

Hereunder the effects of the pteridine derivatives on the lymphocyte activation are elucidated and are compared with standard reference compounds (see table I, compound 4, 6, 7, 11, 13, 19, 20, 21, 22, 25, 26, 28, 30, 34, 35).

Table I summarizes the tested compounds. These pteridine derivatives were obtained as follows:

6-Bromomethyl-1,3-dimethyllumazine:

To a solution of 1,3,6-trimethyllumazine [1] (2.06 g, 0.01 moles) in glacial AcOH (60 ml) was added dropwise bromine (3.2 g, 0.02 moles) in AcOH (10 ml) and then heated under reflux for 1 hour. After cooling was evaporated, the residue dissolved in $CHCl_3$ (100 ml), washed with H_2O (3 x 70 ml), the organic layer dried over Na_2SO_4 and again evaporated. The residue was purified by silica gel column chromatography starting with toluene/EtOAc 9/1 to elute first 6-dibromomethyl-1,3-dimethyllumazine (1.49 g, 41%) and followed by toluene/EtOAc 4/1 to get 6-bromomethyl-1.3-dimethyllumazine. Yield: 1.2 g (42%). M.p. 228°C (decomp.). UV (MeOH): 244 (4.16); [264 (400)]; 337 (3.86).

7-Bromomethyl-1,3-dimethyllumazine:

Analogous to the preceding procedure with 1,3,7-trimethyllumazine [1] (2.06 g, 0.01 moles) and bromine (3.2 g, 0.02 moles) by heating for 2 hours. Isolation by silica gel column chromatography with toluene/EtOAc 9/1 to elute first 7-dibromomethyl-1,3-dimethyllumazine (2.07 g, 57%) and second 7-bromomethyl-1,3-dimethyllumazine. Yield: 0.97 g (34%). M.p.165-166°C. UV (MeOH): 241 (4.23); 338 (4.02).

1,3-Dimethyllumazin-6-ylmethyl-triphenylphosphonium bromide:

To a suspension of 6-bromomethyl-1,3-dimethyllumazine (1.0 g, 3.5 mmoles) in toluene (20 ml) triphenylphosphane (1.1 g, 4.2 mmoles) was added and then heated at 80°C in an oilbath with stirring for 8 hours. After cooling the precipitate was collected, washed with EtOAc and dried at 100°C to give 1.8 g (94%) of a colorless powder of m.p. 289°C. UV (MeOH): 204 (4.74); 227 (4.52); [243 (4.42)]; [262 (4.21)]; 338 (3.88).

1,3-Dimethyllumazin-7-ylmethyl-triphenylphosphonium bromide:
Analogous to the preceding procedure from 7-bromomethyl-1,3-dimethyllumazine and triphenylphosphane in toluene by heating under reflux for 1 day. Yield: 1.86 g (97%). M.p. 261°C. UV (MeOH): 204 (4.76); [221 (4.54)]; 342 (4.09); 414 (4.38).

General synthesis of 1,3-dimethyl-6-(E)-styryllumazines 1, 2, 3, 5:
To a solution of 1,3-dimethyllumazin-6-ylmethyl-triphenylphosphonium bromide and 1,3-dimethyllumazin-7-ylmethyl-triphenylphosphonium bromide (0.547 g, 1 mmole), respectively, in MeOH (5 ml) was added sodium methoxide (0.108 g, 2 mmoles) and stirred at room temperature for 30 min. Then 1.5 mmoles of the aromatic or heteroaromatic aldehyde were added and stirring continued for 5 hours. The resulting precipitate was filtered off, washed with MeOH and purified by recrystallization from DMF/H_2O to give a yellowish powder.

1,3-Dimethyl-6-(E)-styryllumazine (1):
According to the general procedure with benzaldehyde (0.16 g). Yield: 0.124 g (42%). M.p. 238°C. UV (MeOH): [220 (4.17)]; 308 (4.42); 372 (4.03).

1,3-Dimethyl-6-[(E)-2-(pyrid-3-yl)vinyl]lumazine (2):
According to the general procedure with pyridine-3-carboxaldehyde (0.162 g). Yield: 0.195 g (66%). M.p. 210°C. UV (MeOH): [236 (3.92)]; 308 (4.29); 370 (3.97).

1,3-Dimethyl-6-[(E)-2-(pyrid-4-yl)vinyl]lumazine (3):
According to the general procedure with pyridine-4-carboxaldehyde (0.162 g). Yield: 0.156 g (53%). M.p. 262°C. UV (MeOH): 202 (4.20); [238 (3.92)]; 307 (4.51); 370 (4.20).

6-(1,2-Dibromo-2-phenylethyl)-1,3-dimethyllumazine (4):

To a solution of 1,3-dimethyl-6-(E)-styryllumazine (1) (0.735 g, 2.5 mmoles) in $CHCl_3$ (20 ml) was added bromine (0.8 g, 5 mmoles) dissolved in $CHCl_3$ (5 ml) and then the mixture stirred at room temperature for 4 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 1.067 g (94%). M.p. 176°C. UV (MeOH): 245 (4.18); [260 (4.10)]; 341 (3.83).

1,3-Dimethyl-6-[(E)-4-(phenyl)butadienyl]lumazine (5): According to the general procedure with cinnamaldehyde (0.2 g). Yield: 0.138 g (43%). M.p. 252°C (decomp.). UV (MeOH): 228 (4.02); [244 (3.97)]; 330 (4.66); 389 (4.23).

6-[(E)-2-methoxycarbonylethenyl]lumazine:
To a suspension of methoxycarbonylmethyl-triphenylphosphonium bromide (0.415 g, 1 mmole) in dioxane (3 ml) was added DBU (0.23 g, 1.5 mmoles) and stirred at room temperature for 30 min. Then 1,3-dimethyllumazine-6-carboxaldehyde [1] (0.2 g, 0.91 mmoles) was added and stirring continued for 5 hours. The precipitate was collected, washed with MeOH and dried to give a colorless crystal powder. Yield: 0.158 g (63%). M.p. 211-213°C (decomp.). UV (MeOH): 202 (4.46); [256 (4.14)]; 286 (4.21); 348 (4.08).

6-(1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-dimethyllumazine (6):
To a solution of 6-[(E)-2-methoxycarbonylethenyl]lumazine (0.7 g, 2.53 mmoles) in $CHCl_3$ (20 ml) was added bromine (0.64 g, 4 mmoles) dissolved in $CHCl_3$ (5 ml) and then the mixture stirred at room temperature for 6 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 0.97 g (88%). M.p. 163°C. UV (MeOH): 247 (4.16); [260 (4.08)]; 339 (3.88).

6-(2-Bromo-2-methoxycarbonyl-ethenyl)-1,3-dimethyllumazine (7):

To a solution of 6 (0.1 g, 0.23 mmoles) in dioxane (20 ml) was added DBU (70 MG, 0.43 g, 0.43 mmoles) and then stirred at room temperatur for 2 hours. It was diluted with ethyl acetate (100 ml), washed with H_2O (3 x 50 ml), the organic layer separated, dried over Na_2SO_4 and then evaporated. The residue was treated with MeOH, the solid collected and purified by recrystallization from DMF to give a yellowish powder. Yield: 0.055 g (68%). M.p. 204°C. UV (MeOH): [254 (4.08))]; 285 (4.25); 360 (4.01).

6-Chlorocarbonyl-1,3-dimethyllumazine:

A suspension of 1,3-dimethyllumazine-6-carboxylic acid [2] (3.0 g, 12.7 mmoles) in dry toluene (80 ml) was treated with freshly destilled thionyl chloride (50 ml) under reflux for 3 hours. It was evaporated to dryness, the residue treated with dry ether, the solid collected, washed with ether and dried in a vaccuum desiccator. Yield: 3.13 g (93%). M.p. 262-264°C. UV (dioxane): 256 (4.08); [280 (4.00)]; 333 (4.03).

6-[(2-Acetyl-2-ethoxycarbonyl)acetyl]-1,3-dimethyllumazine (8):

A solution of ethyl acetoacetate ethoxy-magnesium salt [3] (0.8 g, 4 mmoles) in THF (8 ml) was added dropwise to a suspension of 6-chlorocarbonyl-1,3-dimethyllumazine (0.51 g, 2 mmoles) in THF (10 ml) and then the mixture stirred at room temperature for 3 days. It was evaporated and the residue treated with cold 1 N HCl (20 ml, 0-5°C). The precipitate was collected, washed with H_2O and dried in a vacuum desiccator. Purification was achieved by column chromatography (silica gel 3.5 x 12 cm) with $CHCl_3$/MeOH 95/5 and the first main fraction collected. After evaporation was recrystallized from toluene (12 ml) to give colorless crystals. Yield: 0.247 g (36%). M.p. 153-156°C. UV (pH 2.0): 251 (4.09); 293 (4.10); 330 (4.11).

6-[2,2-(Diethoxycarbonyl)acetyl]-1,3-dimethyllumazine (9):

To a solution of ethylmalonate ethoxy-magnesium salt [4] (0.685 g, 3 mmoles) in THF (12 ml) was added 6-chlorocarbonyl-1,3-dimethyllumazine (0.51 g, 2 mmoles) and then the mixture stirred at room temperature for 20 hours. It was evaporated, the residue treated with 1 N HCl (20 ml) and the resulting solid collected. Recyrstallization from EtOH (40 ml) gave yellowish crystals. Yield: 0.585 g (78%). M.p. 124-126°C. UV (pH 2.0): 253 (4.05); 291 (4.08); 332 (4.04).

6-(1-Methoxy-2-methoxycarbonyl)ethenyl)-1,3-dimethyllumazine (10):

A suspension of 6 (0.2 g, 0.46 mmoles) in dry MeOH (8 ml) was treated with a solution of sodium (0.046 g, 2 mmoles) in MeOH (2 ml) at room temperature with stirring for 15 min. Then NH_4Cl (0.1 g) and H_2O (10 ml) were added and the mixture extracted with $CHCl_3$ (2 x 50 ml). The organic layer was dried over Na_2SO_4, evaporated and the residue crystallized from $CHCl_3$/n-hexane. Yield: 0.085 g (60%). M.p. 160°C. UV (MeOH): 204 (4.20); 245 (4.15); 288 (4.23); 350 (3.99).

1,3-Dimethyl-6-[(2-nitro)ethenyl]lumazine (11):

A solution of 12 (0.562 g, 2 mmoles) in pyridine (10 ml) was cooled to 0°C and then acetic anhydride (4 ml) dropwise added. Cooling was removed and the mixture stirred at room temperature for 3 hours. The resulting precipitate was collected, washed with H_2O and dried in a vacuum desiccator to give a chromatographically pure product. Yield: 0.515 g (98%). Crystallization from $CHCl_3$. M.p. 232-234°C. UV (MeOH): [239 (3.63)]; 309 (3.87); 365 (3.95).

6-[(1-Hydroxy-2-nitro)ethyl]-1,3-dimethyllumazine (12):

To a solution of nitromethane (0.61 g, 10 mmoles) and triethylamine (1.44 g, 10 mmoles) in MeOH (20 ml) was

added 6-formyl-1,3-dimethyllumazine [1] (2.0 g, 9 mmoles) and then the mixture stirred at room temperature for 5 hours. The precipitate was collected, washed with MeOH and ether and dried. Yield: 2.22 g (78%). Recrystallization from $CHCl_3$. M.p. 166-167°C. UV (MeOH): 240 (4.38); 336 (3.98); [347 (3.89)].

6-[(1-Ethylthio-2-nitro)ethyl]-1,3-dimethyllumazine (13): To a suspension of compound 11 (0.263 g, 1 mmole) in MeOH (5 ml) and H_2O (5 ml) was added ethylmercaptane (0.093 g, 1.5 mmoles) and DBU (0.2 g) and then the mixture stirred at room temperature for 1 hour. The precipitate was collected, washed and dried. Yield: 0.25 g (77%). M.p. 88°C. UV (MeOH): 203 (4.28); 240 (4.26); [262 (4.05)]; 341 (3.89).

6-Hydroxymethyl-1,3-dimethyllumazine (14) [1]:

1,3-Dimethyl-7-[(E)-2-(pyrid-2-yl)vinyl]lumazine (15): According to the general procedure with pyridine-2-carboxaldehyde (0.162 g). Yield: 0.233 g (79%). M.p. 282-283°C. UV (MeOH): 203 (4.14); 238 (4.23); 312 (3.95); 375 (4.36).

1,3-Dimethyl-7-[(E)-2-(pyrid-3-yl)vinyl]lumazine (16): According to the general procedure with pyridine-3-carboxaldehyde (0.162 g). Yield: 0.195 g (66%). M.p. 264-265°C. UV (MeOH): 208 (4.45); 234 (4.43); [274 (4.09)]; 307 (4.08); 375 (4.48).

1,3-Dimethyl-7-[(E)-2-(pyrid-4-yl)vinyl]lumazine (17): According to the general procedure with pyridine-4-carboxaldehyde (0.162 g). Yield: 0.215 g (73%). M.p. 307-310°C. UV (MeOH): 207 (4.12); 229 (4.01); 282 (3.79); [296 (3.76)]; 372 (4.00).

1,3-Dimethyl-7-[(E)-4-(phenyl)butadienyl]lumazine (18):

According to the general procedure with cinnamaldehyde (0.2 g). Yield: 0.195 g (61%). M.p. 277-287°C (decomp.). UV (MeOH): 239 (3.79); 299 (3.66); 402 (4.15).

7-[1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-dimethyllumazine (19):
To a suspension of 7-[(E)-2-methoxycarbonylethenyl]lumazine (1.79 g, 6.5 mmoles) in $CHCl_3$ (70 ml) was added bromine (0.7 g, 14 mmoles) dissolved in $CHCl_3$ (10 ml) and then the mixture stirred at room temperature for 3 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 2.34 g (77%). Crystallization from EtOAc/n-hexane. M.p. 144-145°C. UV (MeOH): 240 (4.14); 343 (3.90).

7-[(E)-2-methoxycarbonylethenyl]lumazine (20):
To a suspension of methoxycarbonylmethyl-triphenylphosphonium bromide (0.415 g, 1 mmole) in dioxane (5 ml) was added DBU (0.23 g, 1.5 mmoles) and stirred at room temperature for 30 min. Then 1,3-dimethyllumazine-7-carboxaldehyde [1] (0.2 g, 0.91 mmoles) was added and stirring continued for 20 hours. The precipitate was collected, washed with MeOH and dried to give a colorless crystal powder. Recrystallization from DMF. Yield: 0.15 g (60%). M.p. 242-245°C (decomp.). UV (MeOH): 201 (4.21); 225 (4.29); 252 (4.20); 364 (4.11).

7-(1,2-Dibromo-2-phenylethyl)-1,3-dimethyllumazine (21):
To a solution of 1,3-dimethyl-7-(E)-styryllumazine (23) (0.735 g, 2.5 mmoles) in $CHCl_3$ (20 ml) was added bromine (0.48 g, 3 mmoles) dissolved in $CHCl_3$ (5 ml) and then the mixture stirred at room temperature for 3 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield:

1.08 g (95%). M.p. 187-188°C. UV (MeOH): 241 (4.25); 341 (4.06).

7-(1-Bromo-2-phenyl)ethenyl-1,3-dimethyllumazine (22):
To a suspension of 21 (0.2 g, 0.44 mmoles) in dry MeOH (4 ml) was added a solution of sodium (0.05 g, 2.2 mmoles) in MeOH (1 ml) and then the mixture stirred at room temperature for 3 hours. The precipitate was collected, washed with MeOH and dried in vacuum. Yield: 0.117 g (71%). Yellowish powder from DMF. M.p. 245-246°C. UV (MeOH): 243 (4.15); 372 (4.15).

7-Benzyl-1,3-dimethyllumazine:
A solution of 5,6-diamino-1,3-dimethyluracil monohydrochloride [5] (2.06 g, 0.01 mole) in H_2O (50 ml) was treated with benzylglyoxal [6] (2.22 g, 0.015 moles) in EtOH (20 ml) and heated under reflux for 1 hour. It was diluted with H_2O (50 ml) and then extracted with $CHCl_3$ (5 x 100 ml). The organic layer was dried over Na_2SO_4, evaporated and the residue purified by silica gel column chromatography with toluene/EtOAc 10/1. The main fraction was collected, evaporated and crystallized from EtOH. Yield: 1.7 g (61%). M.p. 147-148°C. UV (MeOH): 238 (4.22); 332 (4.07).

1,3-Dimethyl-6-(E)-styryllumazine (23):
According to the general procedure with benzaldehyde (0.16 g). Yield: 0.223 g (76%). M.p. 259-260°C. UV (MeOH): 203 (4.17); 237 (4.11); 379 (4.29).

7-Benzoyl-1,3-dimethyllumazine (24):
A suspension of 7-benzyl-1,3-dimethyllumazine (0.56 g, 2 mmoled) in H_2O (30 ml) was treated with $KMnO_4$ (0.6 g) and heated under reflux for 30 min. After cooling was extracted with $CHCl_3$ (3 x 100 ml), the organic layer dried over Na_2SO_4, filtered and evaporated to dryness. Crystallization from dioxane/H_2O. Yield: 0.5 g (84%). M.p. 190-191°C. UV (MeOH): 233 (4.23); [255 (4.10)]; 347 (3.97).

7-Chloro-1,3-dimethyllumazine (25) [7].

1,3-Dimethyl-7-mercaptolumazine (26) [8].

1,3-Dimethyl-6,7-diphenyllumazine (27) [9].

1,3-Dimethyl-6-phenyl-7-mercaptolumazine (28):
A mixture of 7-hydroxy-1,3-dimethyl-6-phenyllumazine [5] (2.84 g, 0.01 mole) and P_4S_{10} (3.3 g) was heated in pyridine (75 ml) under reflux for 1 hour. After cooling was diluted with H_2O (50 ml) and after standing for several hours the yellow precipiptate (28-pyridinium salt, 3.3 g, 87%). The salt was dissolved in hot H_2O (100 ml) and acidified by HCl to pH 0. The resulting yellow crystals were collected, washed and dried in the oven. Yield: 2.22 g (74%). M.p. 145°C (decomp.). UV (MeOH): 203 (4.37); 227 (4.36); [283 (3.86)]; 370 (4.05).

7-Methoxy-1,3-dimethyl-6-phenyllumazine (29):
A solution 7-hydroxy-1,3-dimethyl-6-phenyllumazine [5] (1.42 g, 0.005 moles) in 0.5 N NaOH (20 ml) and MeOH (10 ml) was treated with dimethyl sulfate (1 ml) and stirred for 1 hour at room temperature. The resulting precipitate was collected, washed and dried in the oven. Yield: 1.26 g (81%). M.p. 194°C. UV (MeOH): 205 (4.53); [240 (4.08)]; 281 (4.22); 343 (4.23).

7-Chloro-1,3-dimethyl-6-phenyllumazine (30):
A mixture of 7-hydroxy-1,3-dimethyl-6-phenyllumazine [5] (2.84 g, 0.01 mole) and NH_4Cl (1 g) was heated in $POCl_3$ under reflux for 36 hours. It was evaporated to a syrup, ice was added and stirred with a glasrod till a precipitate was formed. The solid was collected, washed with H_2O, dried and then recrystallized from MeOH. Yield: 2.36 g (78%). M.p. 180°C. UV (MeOH): 204 (4.47); 249 (4.23); 273 (4.24); 350 (4.05).

6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a):

A solution of 6-benzoyl-1,3-dimethyllumazine (0.2 g, 0.68 mmoles) in dry 1,2-dichloroethane (20 ml) was treated with $AlCl_3$ (0.4 g, 3 mmoles) and freshly distilled anisol (10 ml, 92 mmoles) at room temperature and stirred for 24 hours. Then ice (50 g) was added, the aquous phase extracted with $CHCl_3$ (3 x 50 ml), the organic phase washed with 2%-$NaHCO_3$ solution (50 ml) and H_2O (50 ml), dried over Na_2SO_4 and evaporated in high vacuum to remove excess of anisol. The residue was treated with toluene (50 ml) to obtain a yellow precipitate. Recrystallization from EtOH/H_2O 1/1 gave yellow crystals. Yield: 0.176 g (65%). M.p. 240-244°C (decomp.). UV (MeOH): 254 (4.25); [270 (4.21)]; 406 (4.08).

6-Benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31):

A suspension of 6-benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a) (0.3 g, 0.74 mmoles) in dioxane (40 ml) was treated at room temperature with 1%-$KMnO_4$ solution (10 ml) by dropwise addition with stirring. After 30 min the excess of $KMnO_4$ was reduced by $NaHSO_3$, the MnO_2 filtered off, washed with warm EtOH (3 x 20 ml) and then the united organic phases evaporated to dryness. The residue was purified by silica gel chromatography with $CHCl_3$/MeOH (25/1). The main fraction was collected, evaporated and the solid recrystallized from EtOAc with charcoal. Yield: 0.175 g (59%). M.p. 255-257°C. UV (MeOH): 253 (4.24); 367 (4.10).

6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-phenyllumazine (32a):

Analogous to procedure 31a from 6-benzoyl-1,3-dimethyllumazine (0.2 g, 0.68 mmoles) and benzene (15 ml). Yield: 0.21 g (83%). UV (MeOH): 254 (4.26); 407 (4.12).

6-Benzoyl-1,3-dimethyl-7-phenyllumazine (32):

Analogous to procedure 31 from 6-benzoyl-7,8-dihydro-1,3-dimethyl-7-phenyllumazine (32a)(0.3 g, 0.78 mmoles). Yield: 0.18 g (62%). M.p. 185-187°C. UV (MeOH): 252 (4.39); [290 (4.08)]; 349 (4.16).

7-Methoxy-1,3-dimethyl-6-styryllumazine (33):
To a suspension of compound 4 (0.2 g, 0.44 mmoles) in dry MeOH (6 ml) was added DBU (0.2 ml, 1.34 mmoles) and then stirred at room temperature for 2 hours. The precipitate was filtered off, washed with MeOH and dried in a vacuum desiccator. Yield: 0.134 (94%). Crystallization from DMF. M.p. 271-272°C. UV (MeOH): [232 (4.11)]; 306 (4.36); 375 (4.38).

1-Methyl-6,7-diphenyllumazine (34) [10].

7-Hydroxy-3-methyl-6-phenyllumazine (35) [5].

7-Hydroxy-1,6-diphenyllumazine (36).
To a suspension of 6-diamino-5-nitroso-1-phenyluracil [11] (2.32 g, 0.01 moles) in H_2O (50 ml) and EtOH (20 ml) was reduced catalytically with PtO_2/H_2 in a shaking apparatus till about 450 ml of hydrogen was consumed. The mixture was heated, the catalyst filtered off and the filtrate treated with ethyl phenylglyoxylate (2.5 g, 0.014 mmoles) by heating under reflux for 30 min. The warm solution was acidified by HCl to pH 0 and the resulting precipitate collected after cooling. Recrystallization from DMF. Yield: 2.59 (78%). M.p. 330°C. UV (MeOH): 204 (4.54); [222 (4.37)]; 284 (4.17); 346 (4.25).

7-Hydroxy-3,6-dimethyl-1-phenyllumazine (37) [12]:

7-Hydroxy-6-phenyl-1,3-di-n-propyllumazine (38):
A suspension of 5,6-diamino-1,3-di-n-propyluracil (1.13 g, 0.005 moles) in H_2O (30 ml), EtOH (5 ml) and AcOH (2 ml) was treated with ethyl phenylglyoxylate (1.25 g,

0.007 mmoles) and heated under reflux for 30 min forming a brownish oil. After cooling was acidified by HCl to pH 0 whereby the oil solidified. Filtration and recrystallization from EtOH/H_2O gave yellowish needles. Yield: 1.28 g (75%). M.p. 245°C. UV (MeOH): 212 (4.30); [243 (4.01)]; 284 (4.02); 349 (4.19).

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Materials and methods

Various models may be used for testing an immunosuppressive effect. In vivo, for example, different transplantation models are available. They are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response. In vitro, there exist also various models. The most used are lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the experimental conditions applied. There exist different stimuli for lymphocyte activation:

- coculture of lymphocytes of different species (MLR = mixed lymphocyte reaction): lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically.
- CD3 assay: here there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against the CD3 molecule located on the lymphocyte membrane. This molecule has a costimulatory function. The interaction anti-CD3 (= OKT3)-CD3 results in T-cell activation which proceeds via the Ca^{2+}/calmodulin/calcineurin system and can be inhibited by CyA.
- CD28 assay: here specific activation of the T-lymphocyte goes also via an exogenously added antibody against the CD28 molecule. This molecule is also located on the lymphocyte membrane, and delivers strong costimulatory signals. This activation is Ca^{2+}-independent and thus cannot be inhibited by CyA.

Reagents

All derivatives were dissolved in 0.5 ml DMSO and further diluted in culture medium before use in in vitro

experiments. The culture medium consisted of RPMI-1640 + 10% FCS.

Mixed Lymphocyte Reaction

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or EBV-transformed human B cells [RPMI1788 (ATCC name CCL156)] which strongly express B7-1 and B7-2 were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 μCi [^{3}H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a ß-counter.

The percent suppression of proliferation by drugs was counted using the formula:

$$\text{Percent inhibition} = \frac{(\text{cpm+drugs}) - \text{cpm Cult. Med}}{(\text{cpm-drugs}) - \text{cpm Cult. Med}} \times 100$$

T cell purification

T cells were purified by removing non-T cells. Briefly, monocytes were removed by cold agglutination. The resulting lymphoid cells were further purified by a cell enrichment immunocolumn [Cellect Human T (Biotex, Edmonton, Alberta, Canada)] by a process of negative selection. More than 95% of the B cells were removed with this procedure. After depletion, the resulting T cell preparation was highly purified explaining these cells could not be activated by PHA or rIL-2 alone at concentrations capable of stimulating RBMC prior to deletion.

Measurements of T cell proliferations induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA

Highly purified T cells (10^6/ml) were stimulated by immobilized anti-CD3 or anti-CD28 mAb in the presence of PMA. Anti-CD3 mAb (CLB-CD3; CLB, Amsterdam, The Netherlands) were fixed on the 96-microwell plates by incubating the wells with 50 μl of mAb solution (1/800 dilution in culture medium). Anti-CD28 mAb (CLB-CD28; CLB, Amsterdam, The Netherlands) 50 μl (1/650 dilution in culture medium) was added directly to the wells. Further, 20 μl PMA (Sigma, St. Louis, MO, USA) solution (final concentration: 0.5 ng/ml) was added. Subsequently, 20 μl of immunosuppressants were added by serial dilution in triplicate wells. Finally 100 μl of the T cell suspension (10^6/ml) was added. After 48-hour incubation at 37°C in 5% CO_2 20 μl BrdU (100 μM solution) (Cell Proliferation Elisa, Boehringer-Mannheim Belgium) was added to each well. After a further overnight incubation the T cell proliferation was measured using a colorimetric immunoassay for qualification of cell proliferation based on measurements of the incorporation of BrdU during DNA synthesis. The optical density (OD) was measured by a Behring EL311 plate reader at 450 nm (reference wavelength: 690 nm). The percent suppression of proliferation by drugs was counted using the formula:

$$\text{Percent inhibition} = \frac{(\text{OD+drugs}) - (\text{OD Cult. Med.})}{(\text{OD-drugs}) - (\text{OD Cult. Med.})} \times 100$$

<u>In vitro immunosuppressive effect of Pteridine Derivatives as measured with the MLR and with tests involving polyclonal T cell proliferation induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA</u> (table II)

- In the table II column II shows the IC50 values of the various substances in the MLR. The IC50 value represents the lowest concentration of the substances that resulted in a 50% suppression of the MLR.
- Column III shows the IC50 value of the various substances for the anti-CD3 mAb + PMA pathway and row IV the IC50 values of the various substances for the

anti-CD28 mAb + PMA pathway.

- As a comparison the values of other immunosuppressants: CsA, FK506, Rapamycin, Leflunomide and Mycophenolic acid methatroxate (MTX) and 5-Fluoro-uracil (5-FU) in table III are given as well.

Whole Blood Assay (WBA)

WBA is a lymphoproliferation assay performed in vitro but using lymphocytes present in whole blood, taken from animals that were previously given test substances in vivo. Hence it reflects the in vivo effect of substances as assessed with an in vitro read-out assay.

Rats: inbred, male 6- to 8-weeks old R/A rats weighing ± 200 g were used as recipients.

Drug administration: Pteridine derivatives were dissolved in DMSO and further diluted with PBS. Products were given orally in different concentrations 2 times a day for 2 days. To perform the experiments, 6-8 hours after the last administration 1 ml of blood is taken by heart puncture after ether anesthesia and anticoagulated with 100 U/ml of preservative free heparine.

Whole Blood Assay: This assay was performed as we described previously [Use of the Methylxanthine Derivatives A802715 in Transplantation Immunology. II In vitro Experiments. (Yuan Lin, et al., Transplantation 1997, 63, No. 12, 1734-1738)].

Heparinized whole blood was diluted (1:25) with complete RPMI medium and stimulated with 15 μg/ml of concanavalin A (Con A) in triplicate wells in 96-well microtiter plates at 37 °C and 5% CO_2. After 96-h culture, proliferation was determined by measuring the incorporation (cpm) of $[^3H]$-thymidine.

The Con A induced proliferation of lymphocytes taken from rats receiving the test substances (exp) was compared with that from rats receiving only the solvent (con). The percent suppression was calculated as follows:

Results

No.	%suppression	Administration of drugs	Blood taken after:
28 34	57% 63%	80 mg/kg/d 2x/d 2d 40 mg/kg/d 2x/d 2d	8 h 8 h

First, most of the pteridine classes (I) according to the invention contain compounds with a clear suppressive effect in the MLR (mixed lymphocyte reaction). The MLR is considered as an in vitro analogue of the transplant rejection as it is based on the recognition of allogeneic MHC (major histocompatibility antigens) on the stimulator leucotyes, by responding lymphocytes. Various established immunosuppressive drugs are known to suppress the MLR, and were also shown in this description.

From these data it can be deduced that the pteridine derivatives are effective in clinical situations where other immunosuppressants are active as well.

These include the prevention and/or treatment of organ transplant rejection, the prevention and/or treatment of both rejection and the occurrence of graft-versus-host-disease after BM transplantation; the prevention and/or treatment of autoimmune diseases including diabetes mellitus, multiple sclerosis, glomerulonephritis, rheumatoid arthritis, psoriasis systemic diseases such as vasculitis; scleroderma, polymyositis, autoimmune endocrine disorders (thyroiditis), ocular diseases (uveitis), inflammatory bowel diseases (Crohn's disease, colitis ulcerosa), autoimmune liver diseases (autoimmune hepatitis, primary

biliary cirrhosis) autoimmune pneumonitis and auto-immune carditis.

Whereas cyclosporine A and FK506 are only active in the anti-CD3 + PMA test, the pteridine derivatives according to the invention were active, not only in the anti-CD3 + PMA but also in the anti-CD28 + PMA test. It has been shown that the latter is Ca-calmodulin resistant, and resistant to CsA and FK506. The anti-CD28 + PMA pathway has also been called the cosignal pathway and is important to induce energy and even tolerance in T cells. Moreover, representative compounds have been found to be active in an whole blood assay.

Under the term "organ" in the description is understood all organs or parts of organs (even several) in mammals, in particular humans, for example kidney, heart, skin, liver, muscle, cornea, bone, bone marrow, lung, pancreas, intestine or stomach.

After organ transplantation, rejection of the transplanted organ by the recipient occurs (host-versus-graft reaction). After bone marrow transplantation, also rejection of the host by the grafted cell may occur (graft-versus-host reaction). Rejection reactions mean all reactions of the recipient body or of the transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions.

Auto-immune disorders include, inter alia, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, pemphigus, atopic dermatitis, myositis, multiple sclerosis, nephrotic syndrome (in particular glomerulonephritis), ulcerative colitis or juvenile diabetes.

An additive or synergetic effect of pteridine derivatives and other immunosuppressants may be

anticipated. This may be especially, although not exclusively the case for combinations with CyA or FK 506 as the latter are not suppressive in the aCD28 pathway of T cell activation (table III) whereas most pteridine derivatives are.

The invention further relates to the use of cyclosporin A or FK506 or Rapamycine and at least one pteridine derivative according to the invention for the production of a pharmaceutical composition for inhibiting the replication of viruses such as picorna-, toga-, bunya-, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, varicella-zoster-virus or human immunodeficiency virus (HIV); or for treating of cancer such as lung cancers, leukaemia, ovarian cancers, sarcoma, Kaposi's sarcoma, meningioma, colon cancers, lymp node tumors, glioblastoma multiforme, prostate cancers or skin carcinoses.

The invention further relates to the use of cyclosporin A or FK506 or rapamycin and at least one pteridine derivative of the general formula (I) for the production of a pharmaceutical composition for the treatment of human after organ transplantation or of (auto)immune disorders.

Hence, the advantage to associate pteridine with other immunosuppressants may be that, first, the therapeutic spectrum of action of the individual components is quantitatively and qualitatively broadened. Secondly that it allows, by means of a dose reduction without reduced efficacy but with increased safety, that the treatment of immune disorders which were hitherto no indication for immunosuppressive therapy as a result of side effects may be considered. At the same time, the therapy costs can be decreased to an appreciable extent.

As a comparison, known pteridine derivatives are submitted to the same test conditions as the pteridine derivatives of the invention. These compounds and the results thereof are given in table IV and show no

particular immunosuppressive activity.

In all tables

0: concentration > 151 μM

+: concentration range 16-150 μM

++: concentration range 1-15 μM

+++: concentration range < 1 μM

The skilled person will appreciate that the preparation according to the invention may contain the pteridine compounds over a broad content range depending on the contemplated use of the preparation. Generally, the content of the preparation is within the range of 0.01-50 wt.%, preferably within the range of 0.01-10 wt.%, more preferably within the range of 0.1-10 wt.%, and most preferably within the range of 0.1-5 wt.%. Accordingly, the preparation may be used in a dosing regime which is suitable for most contemplated pharmaceutical utilities.

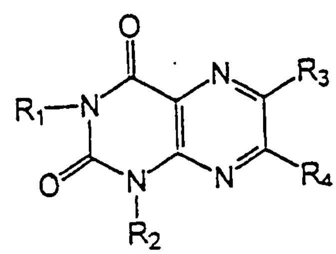
The preparation according to the invention may be used as such or in combination with any acceptable carrier material, excipient or diluent.

The preparation according to the invention may be administared orally or in any other suitable fashion. Oral administration is preferred and the preparation may have the form of a tablet, aqueous dispersion, dispersable powder or granule, emulsion, hard or soft capsule, syrup, elixir or gel. The dosing forms may be prepared using any method known in the art for manufacturing these pharmaceutical compositions and may comprise as additives sweeteners, flavoring agents, coloring agents, preservatives and the like. Carrier materials and excipients may include calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, binding agents and the like. The preparation may be included in a gelatin capsule mixed with any inert solid diluent or carrier material, or has the form of a soft gelatin capsule, in which the ingredient is mixed with a water or

oil medium. Aqueous dispersions may comprise the preparation in combination with a suspending agent, dispersing agent or wetting agent. Oil dispersions may comprise suspending agents such as a vegetable oil.

CLAIMS

1. Pharmaceutical composition for the treatment of autoimmuno disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula:



wherein:

R_1 and R_2 are independently hydrogen; aliphatic saturated or unsaturated; straight or branched carbon chain with 1 to 7 carbon atoms; substituted or unsubstituted aryl or alkylaryl substituents, whereby the carbon atoms may be oxidized represented by alcohol or carbonyl function or carboxylic acids and their esters;

R_3 and R_4 are independently hydrogen, hydroxyl, halogen,alkyl, haloalkyl, alkoxy, wherein alkyl and the alkyl group may be branched or straight and contains one or four carbon atoms, formyl and derivatives such as hydroxylamino conjugates and acetals, cyano, carboxylic acids and carboxyl acid derivatives such as esters and amides, sulfhydryl, amino, alkylamino, cycloalkylamino, alkenylamino, alkynylamino, benzylamino, hydroxylalkylamino, morfolinoalkylamino, fenylhydrazino, morfoline, piperidine, mercaptobenzyl, mercaptoalkyl, cysteinyl ester, styryl, substituted or unsubstituted aromatic ring; aromatic or heterocyclic substituent substituted with an aliphatic spacer between the

pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, whereby said spacer may contain an alcohol function, carbonyl function, halogen, ether, and may be saturated or unsaturated; branched or straight, saturated or unsaturated aliphatic chain of 1 to 7 carbon atoms which may contain one or more functions chosen from the group comprising carbonyl, alcohol, ether, carboxyester, nitro, thioalkyl, halogen or a pharmaceutically acceptable salt thereof; and

X and Y are independently oxygen or sulfur or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier.

2. Pharmaceutical composition according to claim 1, wherein:

R_1 and R_2 are independently hydrogen, methyl, ethyl, propyl, isopropyl, cyclopropyl allyl and other alifatic and alicyclic saturated and unsaturated substituents with 1 to 5 carbon atoms;

3. Pharmaceutical composition according to claim 1-2,
wherein:

R_4 and R_3 are independently hydrogen; hydroxyl; halogen; alkoxy whereby the alkyl group being one to four carbon atoms; cyano; carboxyl acids and carboxyl acid derivatives such as esters and amides; sulfhydryl; substituted or unsubstituted aromatic or heterocyclic ring having as substituents one or more of following functions chosen from the group comprising: halogen, alkoxy, hydroxy, amino, alcohol, ether, nitro, alkyl, alkenyl, haloalkyl and haloalkenyl, cyano, carboxyl acids and derivatives; aromatic or heterocyclic substituent with an aliphatic spacer between the pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, this

spacer may contain an alcohol function, carbonyl function, carboxyl, nitro, halgen, ether and may be saturated or unsaturated, branched or unbranched; branched or straight, saturated or unsaturated aliphatic chain of 1 to 7 carbon atoms which may contain one or more of following functions chosen from the group comprising: carbonyl, alcohol, ether, acetal, amino, imino, amino acid, carboxylester, cyano, nitro, thioalkyl, halogen; or a pharmaceutically acceptable salt thereof.

4. Pharmaceutical composition according to claim 1-3,

wherein:

R_1 and R_2 are methyl.

5. Pharmaceutical composition according to claim 4, wherein R_4 is hydrogen.

6. Pharmaceutical composition according to claim 5, wherein R_3 is haloethyl or haloethylene substituted by phenyl or carboxylic acid ester; or is ethyl or ethylene substituted by nitro.

7. Pharmaceutical composition according to claim 4, wherein R_3 is hydrogen.

8. Pharmaceutical composition according to claim 7, wherein R_4 is haloethyl or haloethylene substituted by phenyl or carboxylic acid ester; or is chloro.

9. Pharmaceutical composition according to claim 4, wherein R_3 is selected from cyano, carboxylic acid ester or dihalo methyl; and R_4 is selected from chloro, amino C_4-alkyl, aminophenyl, thiomethylphenyl, thiomethyl-carboxylic acid ester.

10. Pharmaceutical composition according to any of the previous claims 1-9, wherein the pteridine derivative is a compound selected from the group comprising:

- 1,3-Dimethyl-6-(E)-styryllumazine (1)
- 1,3-Dimethyl-6-[(E)-2-(pyrid-3-yl)vinyl]lumazine (2)

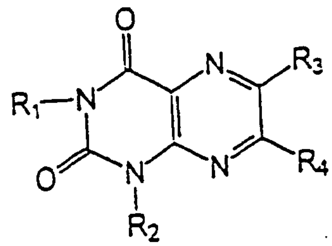
- 1,3-Dimethyl-6-[(E)-2-(pyrid-4-yl)vinyl]lumazine (3)
- 6-(1,2-Dibromo-2-phenylethyl)-1,3-dimethyllumazine (4)
- 1,3-Dimethyl-6-[(E)-4-(phenyl)butadienyl]lumazine (5)
- 6-(1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-dimethyllumazine (6)
- 6-(2-Bromo-2-methoxycarbonyl-ethenyl)-1,3-dimethyllumazine (7)
- 6-[(2-Acetyl-2-ethoxycarbonyl)acetyl]-1,3-dimethyllumazine (8)
- 6-[2,2-(Diethoxycarbonyl)acetyl]-1,3-dimethyllumazine (9)
- 6-(1-Methoxy-2-methoxycarbonyl)ethenyl)-1,3-dimethyllumazine (10)
- 1,3-Dimethyl-6-[(2-nitro)ethenyl]lumazine (11)
- 6-[(1-Hydroxy-2-nitro)ethyl]-1,3-dimethyllumazine (12)
- 6-[(1-Ethylthio-2-nitro)ethyl]-1,3-dimethyllumazine (13)
- 6-Hydroxymethyl-1,3-dimethyllumazine (14)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-2-yl)vinyl]lumazine (15)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-3-yl)vinyl]lumazine (16)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-4-yl)vinyl]lumazine (17)
- 1,3-Dimethyl-7-[(E)-4-(phenyl)butadienyl]lumazine (18)
- 7-[1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-dimethyllumazine (19)
- 7-[(E)-2-methoxycarbonylethenyl]lumazine (20)
- 7-(1,2-Dibromo-2-phenylethyl)-1,3-dimethyllumazine (21)
- 7-(1-Bromo-2-phenyl)ethenyl-1,3-dimethyllumazine (22)
- 1,3-Dimethyl-6-(E)-styryllumazine (23)

- 7-Benzoyl-1,3-dimethyllumazine (24)
- 7-Chloro-1,3-dimethyllumazine (25)
- 1,3-Dimethyl-7-mercaptolumazine (26)
- 1,3-Dimethyl-6,7-diphenyllumazine (27)
- 1,3-Dimethyl-6-phenyl-7-mercaptolumazine (28)
- 7-Methoxy-1,3-dimethyl-6-phenyllumazine (29)
- 7-Chloro-1,3-dimethyl-6-phenyllumazine (30)
- 6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a)
- 6-Benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31)
- 6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-phenyllumazine (32a)
- 6-Benzoyl-1,3-dimethyl-7-phenyllumazine (32)
- 7-Methoxy-1,3-dimethyl-6-styryllumazine (33)
- 1-Methyl-6,7-diphenyllumazine (34)
- 7-Hydroxy-3-methyl-6-phenyllumazine (35)
- 7-Hydroxy-1,6-diphenyllumazine (36)
- 7-Hydroxy-3,6-dimethyl-1-phenyllumazine (37)
- 7-Hydroxy-6-phenyl-1,3-di-n-propyllumazine (38).

11. Pharmaceutical composition containing a pteridine derivative according to claim 1-10 and a compound selected from the group comprising cyclosporine A, substituted xanthines, tacrolimus (FK506), Rapamycin (RPM), Leflunomide, Mofetil, adrenocortical steriods, cytotoxic drugs and antibody compositions as a combined preparation for simultaneous, separate or sequential use in the treatment of auto-immuno disorders or of the prevention and/or treatment of transplant-rejection.

12. Method for treating auto-immuno disorders or transplant-rejections in a subject by administering an effective amount of a pharmaceutical composition of claims 1-11.

13. Compound having the formula:



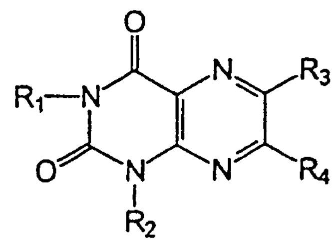
wherein:

R_1, R_2, R_3 and R_4 are defined in claims 1-10.

14. Use of a compound as defined in claim 10 for the treatment of autoimmuno disorders and/or the treatment and/or prevention of transplant rejections.

15. Method for selecting potent immunosuppressive agents based on the determination of the three parameters MLC, ACD_3 and ACD_{28}.

Table I



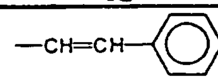
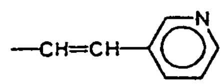
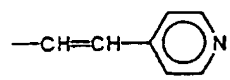
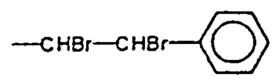
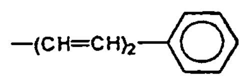
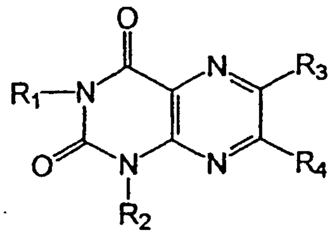
n°	R_1	R_2	R_3	R_4
1	CH_3	CH_3		H
2	CH_3	CH_3		H
3	CH_3	CH_3		H
4	CH_3	CH_3		H
5	CH_3	CH_3		H
6	CH_3	CH_3	$-CHBr-CHBr-COOCH_3$	H
7	CH_3	CH_3	$-CH=CBr-COOCH_3$	H
8	CH_3	CH_3	$-CO-CH(COCH_3)-COOEt$	H
9	CH_3	CH_3	$-CO-CH(COOEt)-COOEt$	H
10	CH_3	CH_3	$-C(OCH_3)=CH-COOCH_3$	H
11	CH_3	CH_3	$-CH=CH-NO_2$	H
12	CH_3	CH_3	$-CHOH-CHNO_2$	H
13	CH_3	CH_3	$-CH(SC_2H_5)-CH_2NO_2$	H
14	CH_3	CH_3	CH_2OH	H

Table I



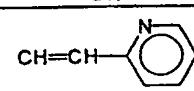
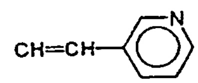
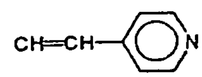
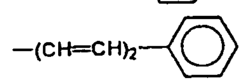
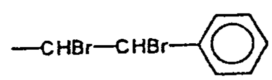
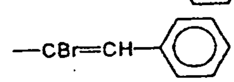
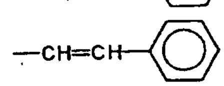
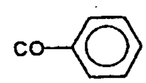
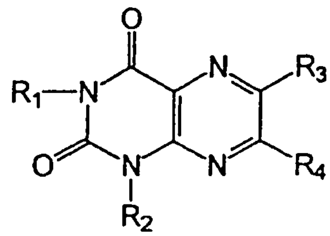
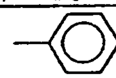
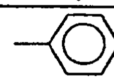
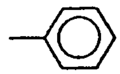
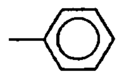
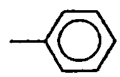
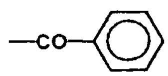
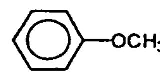
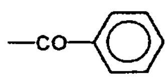
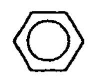
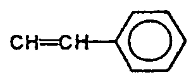
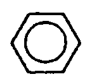
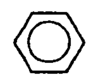
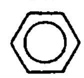
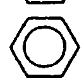
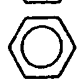
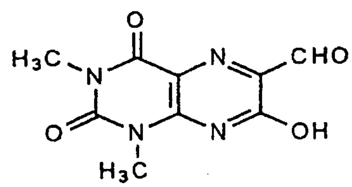
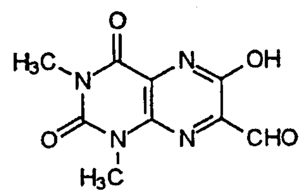
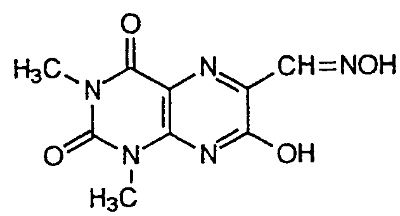
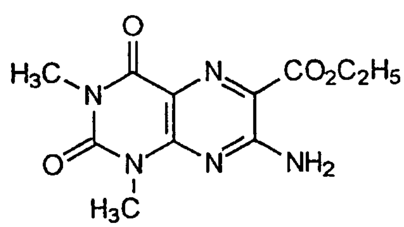
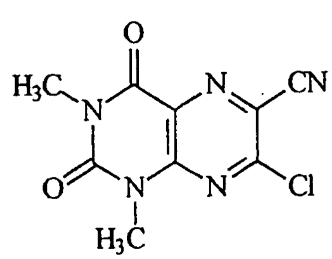
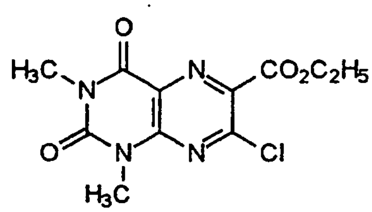
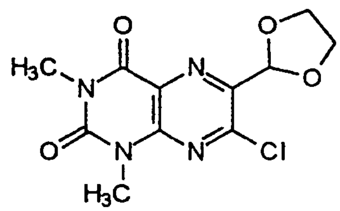
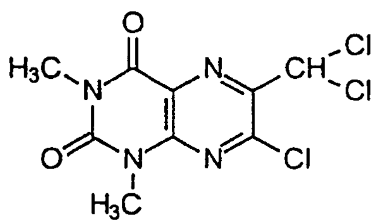
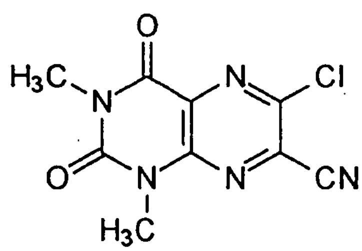
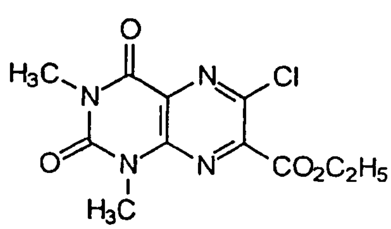
n°	R_1	R_2	R_3	R_4
15	CH_3	CH_3	H	
16	CH_3	CH_3	H	
17	CH_3	CH_3	H	
18	CH_3	CH_3	H	
19	CH_3	CH_3	H	—CHBr—CHBr—$COOCH_3$
20	CH_3	CH_3	H	—CH=CH—$COOCH_3$
21	CH_3	CH_3	H	
22	CH_3	CH_3	H	
23	CH_3	CH_3	H	
24	CH_3	CH_3	H	
25	CH_3	CH_3	H	Cl
26	CH_3	CH_3	H	SH

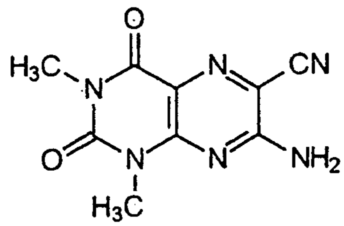
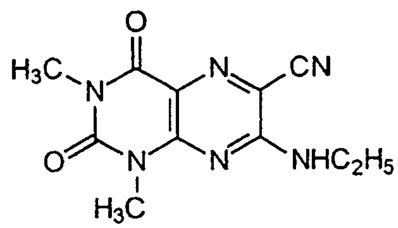
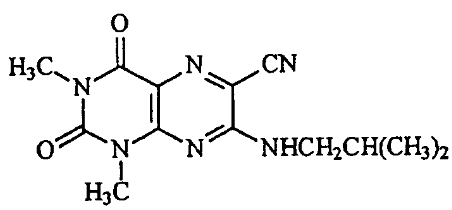
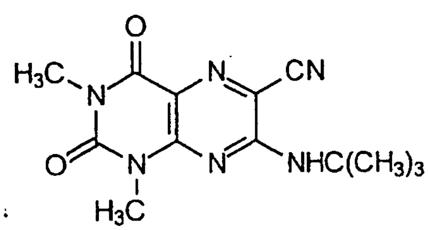
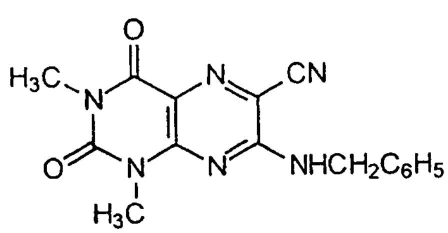
Table I

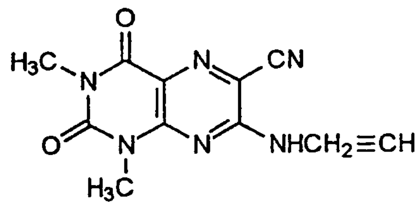
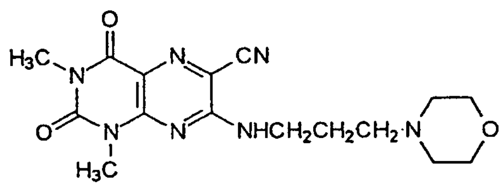
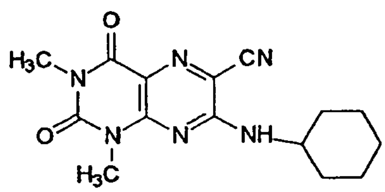
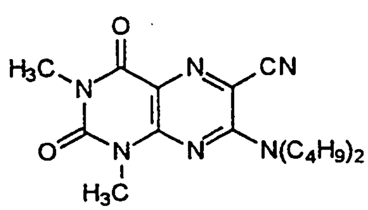
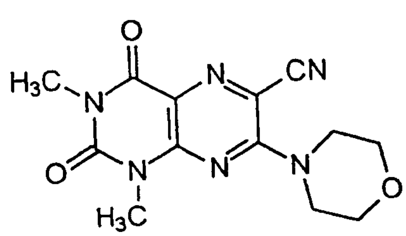


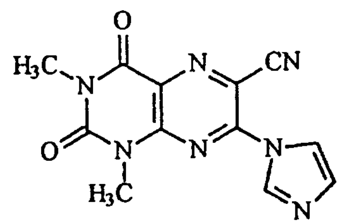
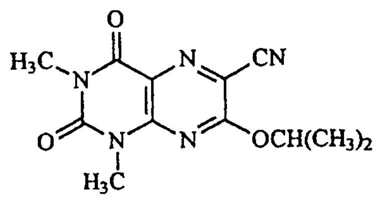
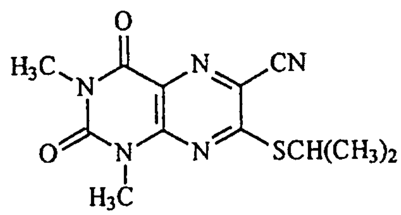
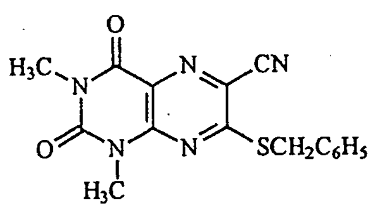
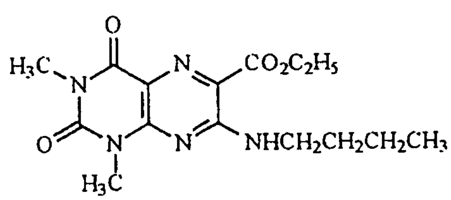
n°	R_1	R_2	R_3	R_4
27	CH_3	CH_3		
28	CH_3	CH_3		SH
29	CH_3	CH_3		OCH_3
30	CH_3	CH_3		Cl
31	CH_3	CH_3		
32	CH_3	CH_3		
33	CH_3	CH_3		OCH_3
34	H	CH_3		
35	CH_3	H		OH
36	H			OH
37	CH_3		CH_3	OH
38	Pr	Pr		OH

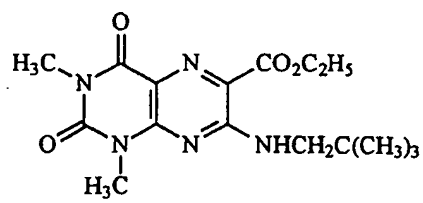
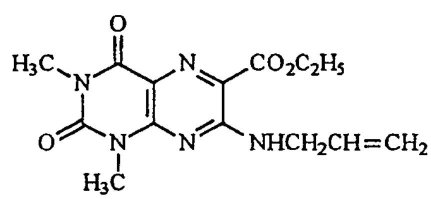
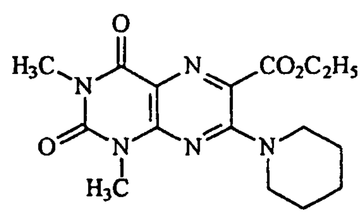
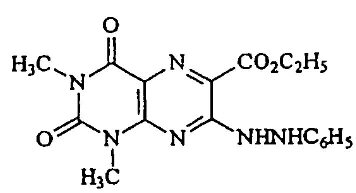
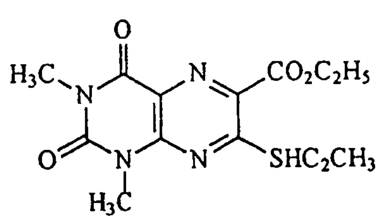
Compound n°		MLR	aCD3	aCD2 8
39		0	++	++
40		+	+	+
41		0	0	0
42		0	0	0
43		+	++	++

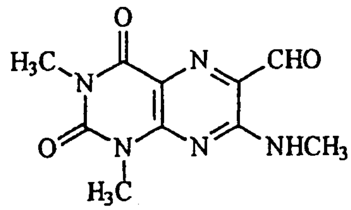
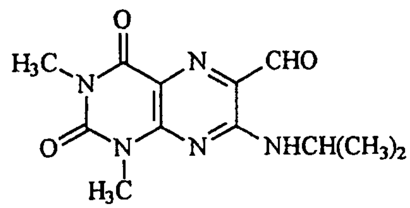
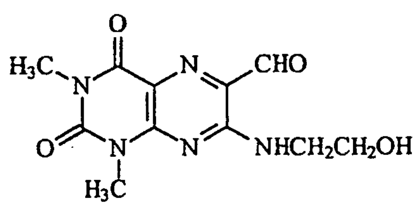
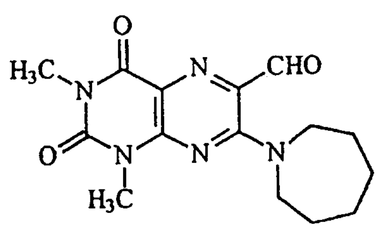
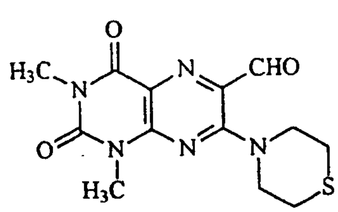
Compound n°		MLR	aCD3	aCD28
44		++	++	++
45		++	++	++
46		++	++	++
47		+	+	+
48		0	0	0

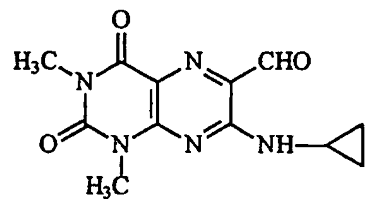
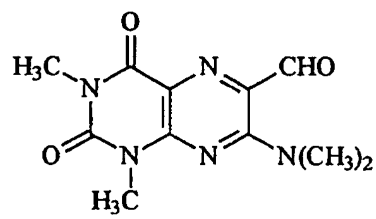
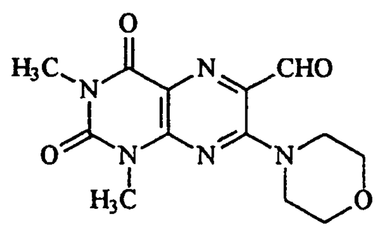
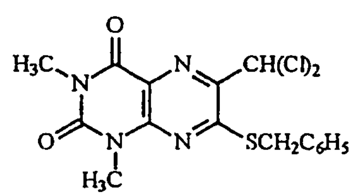
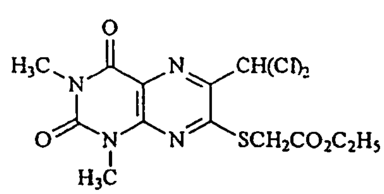
Compound n°		MLR	aCD3	aCD2 8
49		0	0	0
50		0	0	0
51		+	+	+
52		+	+	0
53		+	+	+

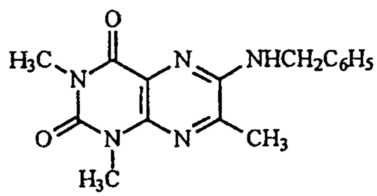
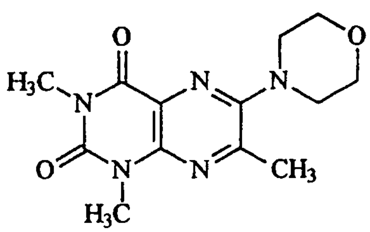
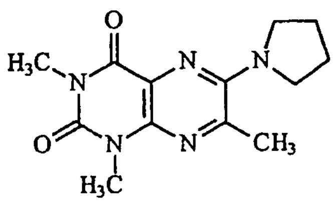
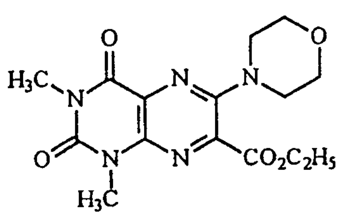
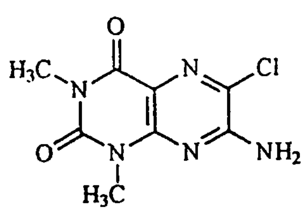
Compound n°		MLR	aCD3	aCD28
54		+	+	+
55		0	0	+
56		++	+	+
57		++	0	+
58		0	0	0

Compound n°		MLR	aCD3	aCD28
59		+	++	++
60		0	0	0
61		+	+	+
62		0	++	++
63		++	+	+

Compound n°		MLR	aCD3	aCD28
64		+	+	+
65		+	+	+
66		+	+	+
67		+	+	++
68		+	+	+

Compound n°	MLR	aCD3	aCD28
69 	+	0	+
70 	+	+	+
71 	+	+	+
72 	+	+	+
73 	+	+	+

Compound n°		MLR	aCD3	aCD28
74		+	+	+
75		0	0	+
76		0	+	+
77		+	++	++
78		+	++	++

Compound n°		MLR	aCD3	aCD28
79		+	+	+
80		0	+	+
81		+	+	+
82		+	+	+
83		0	0	0

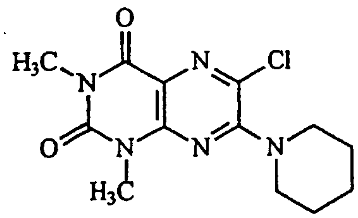
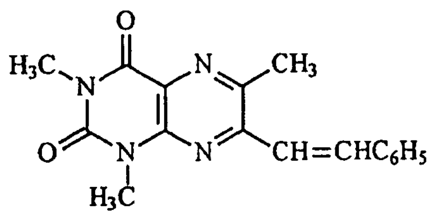
Compound n°		MLR	aCD3	aCD28
84		0	+	+
85		+	+	+

Table II

Compound n°	MLR	aCD3	aCD28
1	75	> 200	> 200
2	30	110	110
3	20	150	150
4	15	3.4	1.55
5	75	140	140
6	12	0.55	0.08
7	4.2	0.58	0.08
8	> 200	80	20
9	> 200	125	90
10	70	80	70
11	12	0.8	0.4
12	90	25	15
13	4.5	0.35	0.09
14	> 200	> 200	25

Table II

IC_{50} in µM of pteridine derivative

Compound n°	MLR	ACD3	aCD28
15	125	125	80
16	20	100	60
17	75	125	100
18	30	110	80
19	1.9	0.5	0.5
20	15	12	12
21	12	0.6	0.1
22	2.7	2.4	2.7
23	50	115	115
24	120	120	80
25	12	0.5	0.1
26	60	35	25

Table II

IC_{50} in µM of pteridine derivative

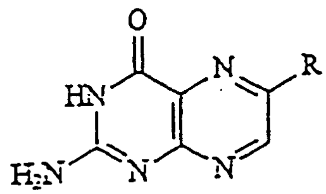
Compound n°	MLR	aCD3	aCD28
27	> 200	85	100
28	25	25	25
29	40	100	70
30	3.75	0.08	0.07
31	150	90	90
32	> 200	75	75
33	20	> 200	> 200
34	35	25	25
35	55	20	25
36	> 200	75	110
37	65	180	> 200
38	120	80	70

Table III

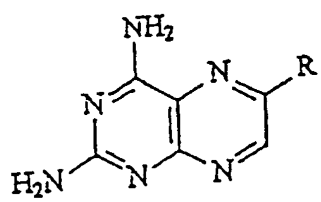
I.S.	IC50		
	Immunosuppressant		
	MLR	aCD3	aCD28
CyA	20 nM	50 nM	N.S.
FK506	1 nM	1 nM	N.S.
Rapamycin	1 nM	1 nM	1 nM
Leflunomide	25 µM	15 µM	20 µM
Mofetil	<0.5µM	50 nM	50 nM
MTX	10 µM	>200 µM	> 200 µM
5-FU		50µM	17 µM

N.S. = not suppressive even not in the highest Concentration

Table IV



R	MLR	aCD3	aCD28
$CH_2OOCOCH_3$	>200	>200	>200
$CH_2OC_6H_5$	>200	>200	>200
CH_2NHCH_3	>200	>200	>200
$CH_2N(CH_3)_2$	>200	>200	>200
$CH_2S\ CH_3$	>200	>200	>200



R	MLR	aCD3	aCD28
$CH_2\ S\ CH_3$	>200	>200	>200
$CH_2\ O\ CH_6(CH_3)_2$	>200	>200	>200
$CH_2\ O\ CH_3$	>200	>200	>200
$CH_2\ O\ CH_2CH_2CH_3$	>200	>200	>200
$CH_2\ NH\ CO\ CH\ (CH_3)_2$	>200	>200	>200